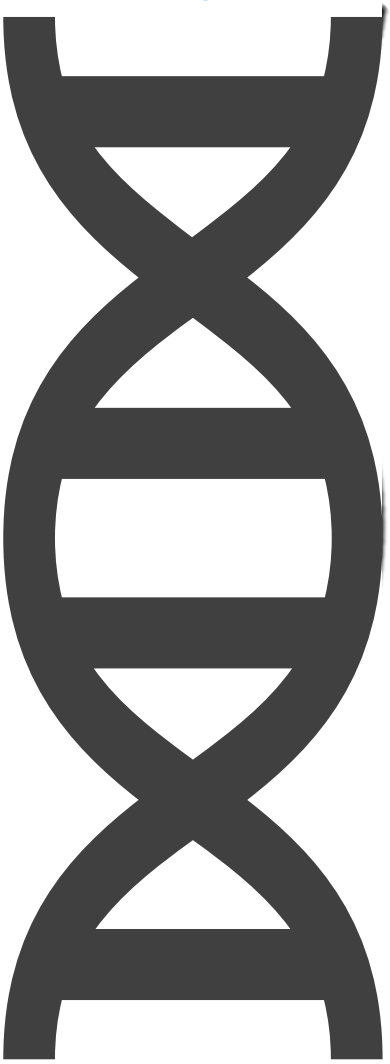
CRISPR Gene Editing



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2021

REVIEW PAPER

Term Paper on CRISPR Gene Editing

Contents

1. Preface

The Nobel prize in chemistry of the year 2020 was awarded to Emmanuelle Charpentier and Jennifer Doudna "for the development of a method for genome editing" [1], namely known as CRISPR-Cas9. As we were curious to know how this technique works and what made it so great, we decided to write our term paper on this topic.

We were especially intrigued by the fact that it can be applied in vivo, meaning in living organisms, with high precision, low costs, and relative ease compared to other methods. As such, we as students asked ourselves how this method works, where it is being applied and what made it so appealing to the scientific community.

2. Introduction

In this paper we will be solely discussing the most commonly used CRISPR technology, which is CRISPR-Cas9. In recent years new types of CRISPR methods which use endonucleases/proteins such as Cas12a, Cas13 etc. have been increasingly investigated due to the limitations of CRISPR-Cas9 to its more widespread application [2].

Recent Events

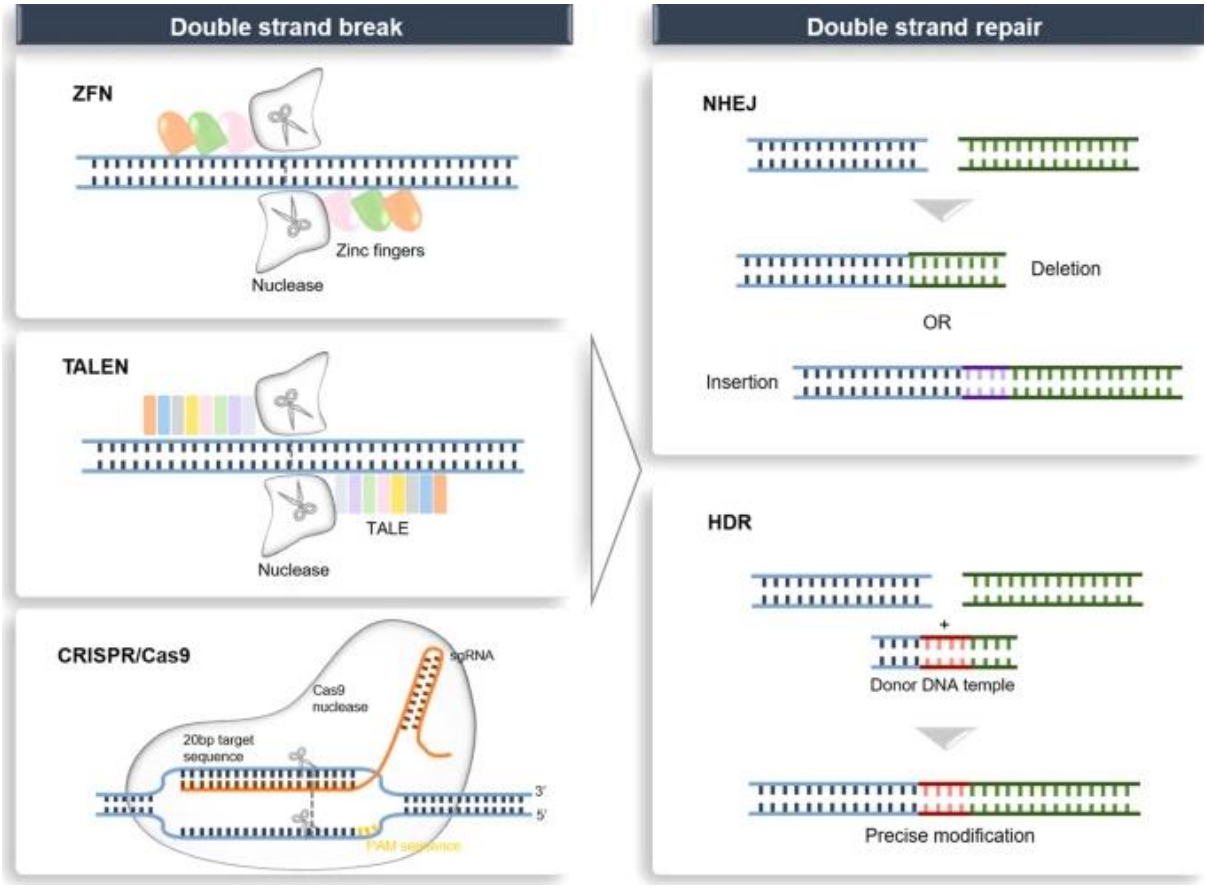
As mentioned above, most recently the Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for the development of CRISPR gene editing.

Additionally, not long ago the first patient ever received gene editing therapy with CRISPR–Cas9 administered directly into their body [3]. A person with a rare condition called Leber congenital amaurosis 10 (LCA10) received the therapy as part of a landmark clinical trial to test the ability of CRISPR–Cas9 gene editing techniques to remove mutations that cause a rare condition. LCA is responsible for the loss of vision during childhood. For this trial, the essential gene editing components were directly injected into the eye.

Ancient Techniques

Over the past decades, three main gene editing techniques have been used, with all three of them using nucleases to target and cut the DNA. Two of these methods, namely meganucleases [4] and transcription activator-like effector nucleases (TALEN) are both completely natural enzymes, eventually modified to target a specific DNA sequence. zinc-finger nuclease (ZFN) [5] however is an artificial endonuclease, made up of a zinc finger protein and a cleavage domain.

They all work by targeting a specific DNA sequence, attaching to it, and inducing a double-strand break (DSB). When the DNA is broken apart, either homologous recombination (HR) or non-homologous end joining (NHEJ) will naturally occur between either two endogenous DNA molecules or between an endogenous and an exogenous DNA molecule.



Comparison of Engineering techniques

Figure taken from [23]

Development of CRISPR

At the end of the twentieth century, the CRISPR sequences were discovered in bacteria, first in E. coli. Since then, CRISPR and the applications of these sequences have been researched. This has led to the discovery of a unique antiviral immune system in prokaryotes and eventually to the CRISPR-associated genome editing technology, firstly presented in 2012 by George Church, Feng Zhang, Jennifer Doudna and Emmanuelle Charpentier. Last year, the latter two earned the 2020 Chemistry Nobel-prize for their discovery, showing what a great step it is for medical and biological fields of research.

Alternative Treatments

Treatments may or may not exist, depending on the disorder one wants to treat. However, treatable disorders like Cystic Fibrosis, often require life-long treatment or one that may not be as effective as a genetic treatment. For example, genetic modifications could be a possible solution to treat some cancers or similar diseases.

For other diseases, there are currently no treatments at all, like for some neurogenetic diseases, which are caused by an “error” in a DNA sequence. These could be cured, or their inheritance could be avoided, by modifying the genes of the person having it, respectively the ones of the baby pre- or postnatally. [6]

3. Description of engineering technique

Before explaining the engineering technique, one needs to understand what CRISPR means. CRISPR stands for Clustered Interspaced Short Palindromic Repeats. One can say these are repeating stretches of DNA i.e., repeating sequences of base pairing, which have unique spacers in between them. Spacers are short segments of sequence that are homologous to phage of plasmid DNA. [7]

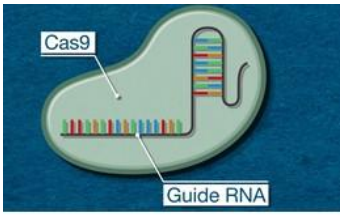


Figure 1:
Components

CRISPRs are found in the genomes of prokaryotic organisms which serve the antiviral defence system of them. CRISPR-Cas9 genome editing, a biomolecular and medical gene engineering technique that may be used to modify genes, is based on this defence mechanism.

Figure 2:
Sequence to be identified

Components

CRISPR is made up of two particularly important components: The Cas9 protein and the gRNA. The two components together form a ribonucleoprotein complex. (Figure 1)

Figure 3:
gRNA corresponding to sequence

Cas9, short for CRISPR associated protein 9, is an enzyme which can cut strands of DNA [2]. To modify the genome, one first needs to cut the existing DNA to be able to change it.

To find the sequence which is to be edited scientist must insert a gRNA, a guide RNA (Figure 3) responsible to locate the exact position of operation. The guide RNA is complementary to the gene sequence in the DNA (Figure 2) and made up of about twenty nucleotides. One can change the genomic target by changing the target sequence present in gRNA. The targeted gene sequence must fulfil one condition: It must be unique compared to the rest of the genome. Otherwise, it could target a not intended region.

Figure 4:
gRNA is attached to Cas9 and complex is introduced to target cells and locates target letter sequence.

gRNA itself is the fusion of two RNA molecules: crRNA and tracerRNA (trans-activating crRNA). Previously, one would bind the Cas9 protein to these two molecules, which would then guide Cas9 to the target site. In 2012, Martin Jinek and colleagues simplified the system by fusing the two components. This helped to transform bacterial CRISPR-Cas9 into a simple, programmable genome-editing tool, which now only required two, namely Cas9 and gRNA. [8]

Figure 5:
DNA can be cut

Concept

To understand the concept of CRISPR technology one can use a simpler example: In a document, if a misspelt word is suspected, one can use the find function to highlight the error and correct it or delete it. Unlike when one uses the find function in a word editing program, one can only search for a single word which corresponds to targeting a sequence which is to be modified in CRISPR as explained above. This find function is taken up by CRISPR/Cas9. It can be thus be defined as a “cut and paste tool for DNA editing" [9].

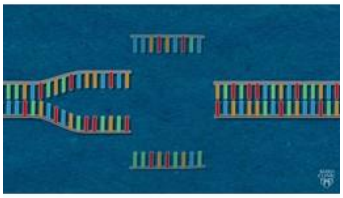


Figure 6:
Genome can be edited by modifying, deleting or inserting new sequences

Figures taken from [9]

Procedure

To use CRISPR/Cas9 one firstly identifies the wanted sequence of in the DNA. Then a specific gRNA is created to recognize this sequence. The gRNA is then attached to Cas9, to make a ribonucleoprotein complex. It is introduced into the target cells, where it locates the target sequence (Figure 4). The Cas9 nuclease can then cut open both strands of the targeted sequence of DNA (Figure 5). Now one can edit the existing genome by modifying existing sequences, deleting existing sequences or inserting new sequences (Figure 6).

When inserting a new DNA sequence and/or re-joining both DNA double-strands together after modifications, two DNA segments need to be recombined. For this important step, two methods exist, namely Homologous recombination (HR) and non-homologous end joining (NHEJ). They both work by using the cell cycle and several proteins. The difference is that HR works by attaching two homologous DNA single strands together, like for DNA replication, whereas NHEJ uses limited to no homology. Thus, HR tends to insert less unwanted modifications. NHEJ however, often deletes or inserts a small unwanted DNA sequence when recombining two DNA double-strands.

4. Interview with an Expert

Due to the current pandemic situation, we were not able to visit any research institutions, unfortunately. That is why we contacted appropriate experts on the subject of CRISPR to conduct an interview. We tried to differentiate between professors who could help us with the scientific aspects and others who could help us with the ethical aspect but unfortunately, we only received answers from Prof. Oliver Bilker. He was open to answer the scientific as well as the ethical questions.

Oliver Billker is a professor at Umeå University in Sweden. His research is about better understanding the biology of the malaria parasite and how they interact with the mosquitoes that transmit them. He uses CRISPR-Cas9 technology to genetically modify malaria parasites.

For the interview, we took time to prepare appropriate questions to which we wished to have the view/opinion of an expert. Our questions were categorised into four topics: Advantages and disadvantages, applications of CRISPR, comparison to other methods, and ethical questions. The entire Interview can be found in the appendix.

Summary of the Interview

Advantages and Disadvantages:
Oliver Billker sees the most potential of the CRISPR method in research and diagnostics. He mentioned that it allowed to deepen the understanding of the biology of organisms and to detect mutations in the genome. He states that new ideas about how to use this technology are emerging all of the time in all domains and that thus its potential is endless. For example, he states that modified crop could be more resistant to herbicides or pests or that some monogenetic diseases in humans could be cured, giving the example of sickle cell anaemia. Still, he puts forward that the development of CRISPR for therapeutical reasons is slower (compared to general research and diagnostic applications) as the risks involved, i.e., the chance of unintended side effects, is greater.

Regarding the disadvantages of this technology, he states that even though presently the precision is accurate, there is always a chance that unintended or unknown changes are made to the genome. Such unintended changes to the genome could cause cancer.

Application and Comparison to other methods:
For Oliver Billker, CRISPR is the most versatile gene editing tool. Also, it is unique because one can easily target specific sequences of DNA in many types of cells. These two qualities give CRISPR the greatest potential to cure genetic diseases compared to other methods. Regarding this and some of the latest news about the clinical use of CRISPR, he is convinced that with good medical infrastructure, genetic modifications can become easier to manage and even be cheaper than conventional treatment.

Ethics:
He states that there are no clear ethical guidelines in scientific domains but that the pros and cons of a modification on an organism must be weight. For example, that he had to make sure, that by modifying pathogenic organisms, they would not become more dangerous. For clinical applications, the question is currently studied but is much more difficult to answer because of the different cultures and the unexpected changes and/or diseases an artificial mutation could cause. He mentions that the partial indistinguishability between artificial genetic modifications and artificial selection regarding plants and animals makes it difficult to agree on a certain rule.

In general, he states that his personal opinion as a scientist is not important, but he believes that CRISPR may be used to alter the genetic material of humans, as long as this modification is done with consent. Finally, he mentions that the question is not whether CRISPR is good or bad, but how it can be used responsibly.

5. Discussion

Over the past decades, other engineering techniques such as ZFN and TALEN were used. These were comparatively slow and risky to apply without creating off-side mutations. CRISPR, however, allows to insert, delete, and modify genes with relative ease while being more cost-efficient and adaptable than the other methods and additionally allowing further studies which would not have been possible prior to the discovery of CRISPR, making it such a revolutionary tool for scientists in medical as well as biological areas.

Currently CRISPR is being used and researched in various fields, ranging from agrotechnology [10] to the creation of biofuel [11], gene therapies against sickle-cell anaemia and beta-thalassaemia [12] and even the possibility of eradicating entire populations trough gene drives [13].

However, there are risks involved. One of the greatest dangers when editing a genome utilising any method, is the unintended and unknown changes made in parts of the DNA which arise through off-target effects, since the targeted sequence might appear in multiple regions of the DNA. Especially, when the double-strand break (DSB) is repaired trough NHEJ or when the DSB is induced at the wrong location. The undesirable mutations then might cause cancer, among other possibilities, due to the now wrong expression of the gene. However, the greater precision of CRISPR reduces the risks of unwanted targeting due to the usage of a gRNA sequence to target the correct sequence on the DNA.

GMOs
Another aspect that must be kept in mind is that the modified organisms which have not been examined to their full extent, for instance newly created genetically modified (GM) crops, could be harmful for the respective ecosystems, if they happen to escape from a lab into nature or when they are used in an open environment. Furthermore, GM species may crossbreed with

another species, forming an unwanted hybrid. This occurrence is known as a type of gene flow. As an example, GM rice passed on its resistance to a herbicide among other benefits onto a weedy rice species [14].

The debate of GM food is of enormous extent, which is why solely some of the most important aspects are discussed here: Among the scientific community there is a consensus that the currently used GM food poses no greater danger to human health than their non-GM counterparts [15]. However, it is believed that they should be assessed on a case-by-case basis before they are introduced to the public [16]. Thus, the opposition is more likely to originate from the public opinion, where some of it is based on a lack of understanding behind the science, as research shows [17].

While GM crops, with traits such as higher yield can help combat world hunger, although not on their own, uncertainty remains. This is based on several factors e.g., the impact on the environment or the possibility of undesired crossbreeds, as mentioned above, and the public opinion, including the fear of monetary profit being the reason for the development of GM crops, instead of a focus on the welfare of humanity. Nevertheless, once such crops have been investigated thoroughly and when handled properly, they may provide prosperity to humankind with a reduced negative impact on the environment and climate.

Also, the regulations on GM foods differ by country: Whilst in some they are allowed be produced, others demand a label on the finished product (if GMOs account for more than 0.9% of the product, such an indication is required in the European Union) or even ban or restrict them. To name a few examples, France and Germany prohibit the use of GM crops, whilst Spain allows the cultivation of MON810 maize, which currently is the only crop authorised for cultivation in the EU.

In general, the European population appears to not be overly concerned about genetically modified organisms, with a “level of concern” of 27% according to a Eurobarometer survey, with a declining trend, especially compared to 2010 where the level was stated to be 69% [18]. Nevertheless, as this subject remains unsolved on an international basis, the controversy on how GMOs should be used remains.

Medicine/Medical Research

CRISPR technology also finds application in medical fields. Whilst currently CRISPR gene editing is not applied on a large scale, it is being researched and experimental trials are already ongoing, e.g., therapies against sickle-cell anaemia, β-thalassaemia [12] and LCA10 [3]. The treatment of various genetically predisposed diseases and disorders is one possible application. There, the greatest potential can be found in treating monogenic disorders, i.e., disorders that arise from a mutation in a single gene. Therefore, the cause for the disorder may be confronted directly, unlike many therapies today which are often based on the reduction of the symptoms. However, a currently faced obstacle is the delivery of the treatment to the affected cells, tissue, and organs, especially when the defective gene(s) are found in a multitude of them.

Additionally, it may be used as a diagnostic or testing tool. For instance, a test which is based on CRISPR-Cas12 for detecting SARS-CoV-2 RNA allows the detection of the virus in respiratory swab RNA extracts in under 40 minutes [19]. This procedure would form an alternative to RT-PCR, as it also would end up cheaper and independent on the varying temperatures required for that method. As of now, such tests may be used in the USA for emergencies [20].

The ability to edit genes also faces opposition in this field, especially regarding the editing of the human germline, i.e., changing the DNA of cells which pass on their genetic material in the form of egg or sperm cells through genetic technology, meaning that these changes of the

genome may be inherited by the offspring. Generally, engineering the human genome is currently viewed as going too far.

Genetically edited Babies

On November 26, 2018, He Jiankui, a Chinese biophysics researcher, announced that he had helped to successfully create the first two genetically altered babies called Lulu and Nana, those names being “code names”, using CRISPR/Cas9 [21]. He informed that the modifications were made in the gene called CCR5 and that this modification would bestow resistance to an HIV-1 infection to the twin girls.

However, after he published this information, he received much backlash from both, the public and the scientific community, which led to ethical and legal controversies and eventually to the conviction of him and his collaborators Zhang Renli and Qin Jinzhou on the 30th of December 2018 for the conduct of illegal medical practices [22].

The negative view towards his experiment is based on ethical, social and security concerns, making such practices therefore illegal and many countries. These include the uncertainty of the accuracy of CRISPR, not understanding the functions of the entire human genome but also the lack of an international framework. Additionally, some argue that each person must have the right to firstly give their consent to have their genes edited by artificial means, a consent that cannot be given before the person is born. Others also believe that the human body is sacrosanct because of their opinion or religion.

Another point is the fear of so called “designer babies”. This name is given to, currently theoretical, children who had their genome modified, so they would have selected traits or phenotypes, such as height, eye colour, enhanced cognitive or physical abilities, higher resistance to various diseases or even a higher age expectancy. Nevertheless, the current state of knowledge is not advanced enough yet to create such “designer babies” without risks. At present, this discussion remains mainly hypothetical but with a critical attitude towards such acts.

The Future of CRISPR

In the future CRISPR technology is believed to have exciting potential for curing many genetically caused disorders in vivo, such as cystic fibrosis and haemophilia or even for overcoming cancer. This technology also will continue being used as a tool for the analysis of the DNA of various organisms and the creation of a multitude of GMOs.

To achieve these goals, further research of the CRISPR/Cas-systems must be conducted and the technology further developed, so that the off-target effects may be reduced, and the efficiency increased. Eventually, a completely new revolutionary technique for editing genes may be developed, but until then CRISPR will remain as one of the most promising methods for changing the genetic material in vitro and in vivo.

6. Summary

CRISPR is a powerful gene editing tool which has immense potential in research, diagnostics as well as therapeutic application. It uses gRNA to precisely locate a DNA sequence and then, it induces a DSB, which is repaired by NHEJ or HR. Not its global working but its composition makes it a unique gene editing tool. Compared to other more ancient technologies, this construction gives it the major advantages to be more precise and faster than them, although it is still being perfectionated nowadays. In a presumably not so distant future, this technology will permit to cure nowadays uncurable or to facilitate the cure of diseases, should they be genetic or not. For an expert in the domain of gene editing and modification, CRISPR has a lot of potential applications, not only in therapeutical domains but also in research, where it allows further investigation to be performed easier and faster than before.

As the technology progresses over the year the scientific community must be aware that it is used for the right ethical purposes. The wrong uses can have major negative consequences which would change the society we live in today. Cases such as the He Jiankui case must be avoided at all costs. The technology should not be in bad hands. One should always remember where science ends and the unethical genome manipulation of future generations begins. Every human has the right to decide whether he or she would like the use of this technology and one should respect that like the hesitancy in vaccinations against COVID-19. Also, as such technologies become available, some philosophical questions of the past become real social questions, like, should we as human manipulate our evolution? Where is the limit between curing a disorder and enhancing a specific feature of our body? These are questions which only the future can answer.

7. References

[1] Nobel Media, “The Nobel Prize in Chemistry 2020,” 7 October 2020. [Online]. Available: https://www.nobelprize.org/prizes/chemistry/2020/summary/. [Accessed 04 February 2021].

[2] A. Vidyasagar, “What Is CRISPR?,” Livescience, 21 April 2018. [Online]. Available: https://www.livescience.com/58790-crispr-explained.html. [Accessed 17 January 2021].

[3] H. Ledford, “CRISPR treatment inserted directly into the body for first time,” nature, 05 March 2020. [Online]. Available: https://doi.org/10.1038/d41586-020-00655-8. [Accessed 17 January 2021].

[4] R. Molina, G. Mentoya and J. Prieto, “Meganucleases and Their Biomedical Applications,” 17 January 2011. [Online]. Available: https://www.researchgate.net/publication/259713992_Meganucleases_and_Their_Biomedical_Applications. [Accessed 3 February 2021].

[5] F. D. Urnov, E. J. Rebar, M. C. Holmes and H. S. Z. &. P. D. Gregory, “Genome editing with engineered zinc finger nucleases,” September 2010. [Online]. Available: https://www.nature.com/articles/nrg2842. [Accessed 3 February 2021].

[6] C. Tachibana, “Sciencemag: Beyond CRISPR: What’s current and upcoming in genome editing,” 27 September 2019. [Online]. Available: https://www.sciencemag.org/features/2019/09/beyond-crispr-what-s-current-and-upcoming-genome-editing.

[7] I. Grissa, G. Vergnaud and C. Pourcel, “The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats,” May 2007. [Online]. Available: https://doi.org/10.1186/1471-2105-8-172. [Accessed 31 January 2021].

[8] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. a. Doudna and E. Charpentier, “A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity,” Science, vol. 337, no. 6096, pp. 816-821, 2012.

[9] Mayo Clinic, “CRISPR Explained,” Youtube, 24 July 2018. [Online]. Available: https://www.youtube.com/watch?v=UKbrwPL3wXE. [Accessed 22 January 2021].

[10] A. P. Taylor, “Companies Use CRISPR to Improve Crops,” TheScientist, February 2019. [Online]. Available: https://www.the-scientist.com/bio-business/companies-use-crispr-to-improve-crops-65362. [Accessed 26 January 2021].

[11] A. Stapleton, “Gene-Editing Algae Doubles Biofuel Output Potential,” science alert, 23 June 2017. [Online]. Available: https://www.sciencealert.com/gene-editing-algae-doubles-biofuel-output-potential. [Accessed 26 January 2021].

[12] H. Ledford, “CRISPR gene therapy shows promise against blood diseases,” nature, 8 December 2020. [Online]. Available: https://www.nature.com/articles/d41586-020-03476-x. [Accessed 26 January 2021].

[13] M. Scudellari, “Self-destructing mosquitoes and sterilized rodents: the promise of gene drives,” nature, 9 July 2019. [Online]. Available: https://www.nature.com/articles/d41586-019-02087-5. [Accessed 26 January 2021].

[14] W. Wang, H. Xia, X. Yang, T. Xu, X. x. C. Hong Jiang Si, F. Wang, J. Su, A. A. Snow and B.-R. Lu, “A novel 5-enolpyruvoylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (Oryza sativa) without herbicide,” New Phytologist (Volume 202, Issue 2), pp. 679-688, 01 August 2013.

[15] P. Ronald, “Plant Genetics, Sustainable Agriculture and Global Food Security,” Genetics, vol. 188, pp. 11-20, 1 May 2011.

[16] World Health Organization, “Food, genetically modified,” 1 May 2014. [Online]. Available: https://www.who.int/news-room/q-a-detail/food-genetically-modified. [Accessed 25 January 2021].

[17] J. McPhetres, B. T. Rutjens, N. Weinstein and J. A. Brisson, “Modifying attitudes about modified foods: Increased knowledge leads to more positive attitudes,” Journal of Environmental Psychology, Volume 64, pp. 21-29, August 2019.

[18] European Food Safety Authority, “Food safety in the EU,” 7 June 2019. [Online]. Available: https://doi.org/10.2805/661752. [Accessed 27 January 2021].

[19] J. P. Broughton, X. Deng, G. Yu, C. L. Fasching, V. Servellita, J. Singh, X. Miao, J. A. Streithorst, A. Granados, A. Sotomayor-Gonzalez, K. Zorn, A. Gopez, E. Hsu, W. Gu, S. Miller, C.-Y. Pan and Hug, “CRISPR–Cas12-based detection of SARS-CoV-2,” 16 April 2021. [Online]. Available: https://doi.org/10.1038/s41587-020-0513-4. [Accessed 1 February 2021].

[20] G. Guglielmi, “First CRISPR test for the coronavirus approved in the United States,” Nature, 8 May 2020. [Online]. Available: https://www.nature.com/articles/d41586-020-01402-9. [Accessed 3 February 2021].

[21] H. Jiankui, “About Lulu and Nana: Twin Girls Born Healthy After Gene Surgery As Single-Cell Embryos,” The He Lab, 26 November 2018. [Online]. Available: https://www.youtube.com/watch?v=th0vnOmFltc. [Accessed 02 February 2021].

[22] D. Normile, “Chinese scientist who produced genetically altered babies sentenced to 3 years in jail,” Science, 30 December 2019. [Online]. Available: https://www.sciencemag.org/news/2019/12/chinese-scientist-who-produced-genetically-altered-babies-sentenced-3-years-jail. [Accessed 2 February 2021].

[23] H. Li, Y. Yang, W. Hong, M. Huang, M. Wu and X. Zhao, “Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects,” Signal Transduction and Targeted Therapyopen, 3 Jauary 2020. [Online]. Available: https://doi.org/10.1038/s41392-019-0089-y. [Accessed 02 February 2021].

[24] W.-D. H. Xuan Li, “Nature: Homologous recombination in DNA repair and DNA damage tolerance,” 1 January 2008. [Online]. Available: https://www.nature.com/articles/cr20081.

8. Appendix

Our Interview with Professor Oliver Billker:

Advantages and disadvantages:
What are the current and potential future domains of application of the CRISPR method?
CRISPR technology is now used widely in research and diagnostics. In research, it helps us understand the biology of organisms, including many pathogens, that could not previously be studied because our tools were too inefficient. In diagnostics, Cas9 can be used to detect pathogen DNA or cancer mutations. Tests for coronavirus or malaria are already available, for instance. I think its use for engineering genomes of crop plants to increase, for instance, yield or resistance to herbicides or pests is well underway. There is great potential for using the technology as a therapy for many monogenetic diseases in humans. These are diseases that are caused by a single, known mutation. Examples include sickle cell disease, cystic fibrosis, Duchenne muscular dystrophy, Huntington's, or haemophilia. Experimental cancer treatments are also in the pipeline, where scientists try to engineer a patient's immune cells to turn them against their tumour cells. I think every scientist in the biological and medical sciences is asking what CRISPR can do for them and new ideas are emerging all the time.

In which domains do you see the greatest possibilities?
Currently, the impact as a research tool is phenomenal. In future, I believe there will be a great impact on crops and as disease therapy.

What would you consider the largest disadvantage of the CRISPR method?
The precision of CRISPR is very good but no biological tool is perfect. There can be unintended or unknown consequences when CRISPR/Cas9 changes parts of the genome it wasn't meant to target. This can happen at a low rate.

Are there be any known but unwanted side effects when applied to living organisms?
"Side effects" would be unintended effects of therapeutic applications of the technology. These could come from unintended (off-target) mutations introduced by the technology, which are feared could cause cancer. It is also possible that the intended modification has unintended consequences. All this needs to be carefully considered. Off-target effects need to be assessed and their potential to cause cancer needs to be evaluated. I am no expert in this area, but I think the need to move carefully is what determines the relatively slow rate of progress in applying CRISPR-Cas9 therapeutically (as compared to research applications).

Are there any concerns regarding the safety of this gene editing technology?
Therapeutic applications in humans target cells that are somatic, i.e. they are cells that do not give rise to eggs or sperms. Such mutations cannot be passed on to the next generation. Modification of the germline is much more controversial.

Another consideration we need to keep in mind when editing genomes is whether modified genetic sequences can accidentally escape the laboratory, or if their release through crop plants and farm animals is intended, whether they can spread into wild animals or plant populations.

Application and comparison to other methods:
Is the CRISPR method really so different compared to the more ancient methods e.g. HE or ZFE?
CRISPR is unique in that it can easily be programmed to target almost any sequence. No other tool is as versatile. It also works in many species and cell types.

Could the CRISPR method be used industrially?
Hm, not sure what you mean. It is certainly used already commercially in many different ways. For instance, there are companies offering services for the research community using CRSPR or producing diagnostics. Many companies do research and clinical trials for therapeutic applications or to generate and test genetically modified crop plants.

Do you think CRISPR will be the major technology to cure lifelong genetically inherited diseases in the future?
It has currently the greatest potential.

Do you think CRISPR gene editing will be accessible to 'everyone' in the future by lowering the currently high cost and labour associated with gene editing?
I saw a news item just last month on the first clinical use of genetic therapies for sickle cell disease and beta-thalassaemia: https://www.nature.com/articles/d41586-020-03476-x. The results look promising. If the therapeutic effect lasts for years or a lifetime, I would guess that this therapy is already cheaper and better for patients then managing the disease with conventional approaches. It does require a well-developed health care infrastructure providing a good level of care. The therapy couldn't currently be rolled out in Africa, I think, where sickle cell disease is most common.

Ethical questions:
What is the current ethical guideline on how CRISPR can be used?
There are no guidelines specifically regulating CRISPR. There are general rules according to which the biological safety of genetically modified organisms needs to be assessed by researchers before they undertake their work. For instance, if I want to modify the genome of an organism in the laboratory I need to make sure that the modified organism cannot escape. If I modify the genome of a research animal, I need to consider the possibility that this causes suffering, in which case I need to be sure this is unavoidable, keep it to the absolute minimum necessary to do the research, and that the benefits that will come from the research will outweigh the costs of the suffering it causes. When modifying the genome of a pathogenic microorganism, I need to consider whether this will make the pathogen more dangerous and what precautions I need to put in place in case it does.

Furthermore, there are ethical review processes for clinical interventions. The bar is very high for taking gene therapies into humans.

CRISPR also throws up important ethical questions that in the past were discussed theoretically but now requires decisions in real life. In many cases, we do not have a consensus in society on how to answer them. Should it be allowed at all to modify the human germline? For which reason? Can and should we define a line between preventing debilitating genetic disease and so-called designer babies?

Another interesting point is that CRISP editing can create or reverse mutations in a way that the outcome cannot be distinguished from a natural mutation that occurs in any population at a certain (low) rate. A breeder of plants or domestic animals works by selecting from the genetic variants that occur naturally in a population. If we can create exactly the same trait (larger apples, pathogen resistant potatoes) and the same genomic sequence by engineering into a genome what also exists in nature, such that the resulting genomes after breeding and engineering are indistinguishable, why should different rules apply? I am not saying I have an answer to this.

Also, note that ethical rules and concepts differ between countries and cultures.

If CRISPR becomes widely available in the future, do you believe that it should be allowed to use this technology to enhance basic traits such as athletic ability, height, and appearance of new-borns?
Ethical questions need to be decided by society and require a broad consensus. Scientists are no better or more relevant than anybody else in making these decisions. This is why all ethics committees have lay people on them, i.e. individuals who are not experts. It is the role of the scientists to present all the facts in a non-technical manner, so everyone can appreciate and evaluate the ethical consequences of the decisions we take as a society, which are reflected in the laws our elected representatives make.

My personal opinion is therefore not important. I do not want to evade the question, though. I personally do not think that genomes are sacrosanct. Interfering with someone's genome, with their consent, in a particular tissue or cell type to cure them of a debilitating disease is a good thing, if side effects have been carefully evaluated and future generations are not affected in an unpredictable manner.

Germline modifications raise more serious questions of human dignity and our impact on future generations. I haven't thought deeply about this. Now, that the technology to modify the germline is here, we need to remind ourselves that there are clear rules not to use it. This line has already been crossed by one group of scientists (look up He Jiankui to learn more), who alleged to have engineered an HIV resistant baby. Their work was condemned as illegal and unethical, and He Jiankui had to bear the consequences.

It is not a question of CRISPR being a good or a bad thing. With any technology, the question is how to use it responsibly. I see no compelling reason why we should modify the human germline. I am also not convinced current societies would deal well with the responsibility for future generations if the option was available.